THE SENECIO ALKALOIDS

The correlation of structures with particular reference to senecic acid and its isomers.

By

Giovanni Parazzi, B.Sc.(Gen.Hons.)London.

A thesis presented to The University of Cape Town in partial fulfilment of the requirements for the degree of Master of Science.

Department of Chemistry,
University of Cape Town,
C.S.I.R. Natural Products Research Unit,
CAPE TOWN.

1967.

A C K N O W L E D G E M E N T S

The author wishes to express his sincere thanks to:-

Professor F. L. Warren for his invaluable guidance and encouragement in this work.

Mrs. G. Y. Merchant for the micro-analysis.

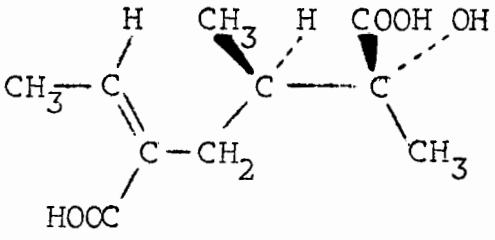
Mrs. J. Oates for typing and stencilling the formulae in this thesis.

The C. S. I. R. for their research grant which made this work possible.

C O N T E N T S

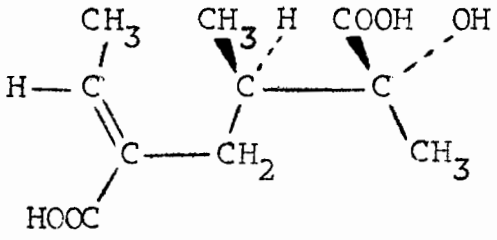
S U M M A R Y

Senecic and integerrinecic acid have been assigned structures (A) and (B) on the basis of degradation reactions and X-ray studies.



(A)

Senecic acid



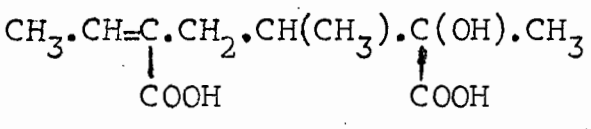
(B)

Integerrinecic acid

Two isomers arise, namely; platynecic and usaramoensenecic acid and are reported in the literature. Their structure as isomers of (A) and (B) have been speculated.

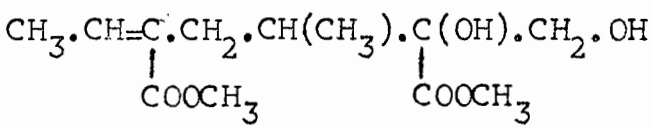
This thesis is concerned with a study of the isomers of (A) and (B) and leads to the conclusion that platynecic and usaramoensenecic acid do not exist and are probably mixtures.

The procedure for this study involved the oxidation of isatinecic (C) and retronecic (D) acids and of their esters to the keto derivative (E) and a Grignard reaction to give (F) which can be one of the isomers of (A) and (B).



(A) cis form

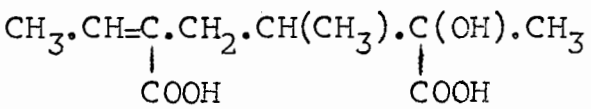
(B) trans form



(C) cis form

(D) trans form

keto acid



(F)

(E)

INTRODUCTION

INTRODUCTION

a) The alkaloid structure.

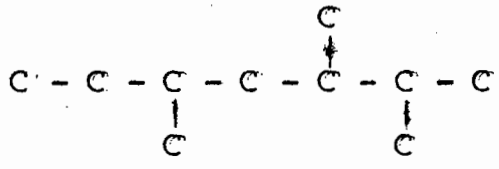
The Senecio alkaloids, in general, comprise a group of basic substances which have a methyl-pyrrolizidine nucleus with one or more hydroxyl groupings esterified by organic acids. The acids are obtained from the alkaloid by hydrolysis or hydrogenolysis and can be classified into three groups, i. e. : (1) C_{10} adipic or "necic" acids.

(2) Glutaric acids.

(3) C_7 acids.

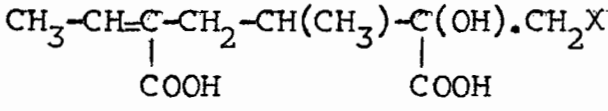
b) The C_{10} adipic or "necic" acids.

The "necic" acids are terpenic acids, as was originally suggested by Barger in 1936 and later proved by Kropman and Warren[1] who also showed that four of these acids had the same irregular isopreme skeleton:



(I)

The general formula for these four acids is as follows:-



(II)

where X = OH in Isatinecic acid (cis)

Retronecic acid (trans)

X = H in Senecic acid (cis)

Integerrinecic acid (trans)

Two more acids have also been claimed to have the same general structure and formula as senecic acid: usaramoensenecic[2] and platynecic acid[3].

These latter acids have not been re-isolated and were not reported in the synthesis of senecic acid carried out by Culvenor and Geissman[4]. A study of the formula (II) shows that there are two asymmetric carbon centres and a pair of double bonds within the same carbon skeleton and this can give rise to eight isomers. The author has accordingly attempted to correlate the stereo-chemical relationship of these acids containing this uniform structure.

c) Attempts to correlate the stereo-chemistry of these acids.

Isatinecic and retronecic acids were shown by Christie, Kropman and Warren[5] to be cis- and trans-1:2-dihydroxy-3-methylhept-5-ene-2:5-dicarboxylic acids respectively, with the geometric isomerism about the double bond giving rise to the two acids.

Senecic acid, which occurs in a number of alkaloids as shown on Table I, was analysed by Kropman and Warren[6] who showed that it was a 2-hydroxy-3-methyl-5-ene-2:5- . dicarboxylic acid. The lactone of senecic acid, as demonstrated by Kropman and Warren[7], gave the trans-senecic acid which was identified as integerrinecic acid obtained by Manske[8]. The geometrical isomerism of senecic acid and integerrinecic acid thus parallels that found for isatinecic and retronecic acids.

Table I. Occurences of Senecic acid.

Alkaloid	Base	Acid	Ref.
Platyphylline	Platynecine	Senecic or Platynecic acid	25 26 3
Rosmarinine	Rosmarinecine	Senecic acid	27 28
Renardine	Otonecine	Senecic acid	29
Senecionine	Retronecine	Senecic acid	30 31
Senkirkine	?	Senecic acid lactone	32
Neoplatyphylline	Platynecine	Isomers of Senecic acid	33

Culvenor and Geissman[4] synthesized integerrinecic and senecic acid as shown on Chart I, and in doing so showed the absolute geometry of the compounds in the light of X-ray studies recently performed by Fridrichson[9].

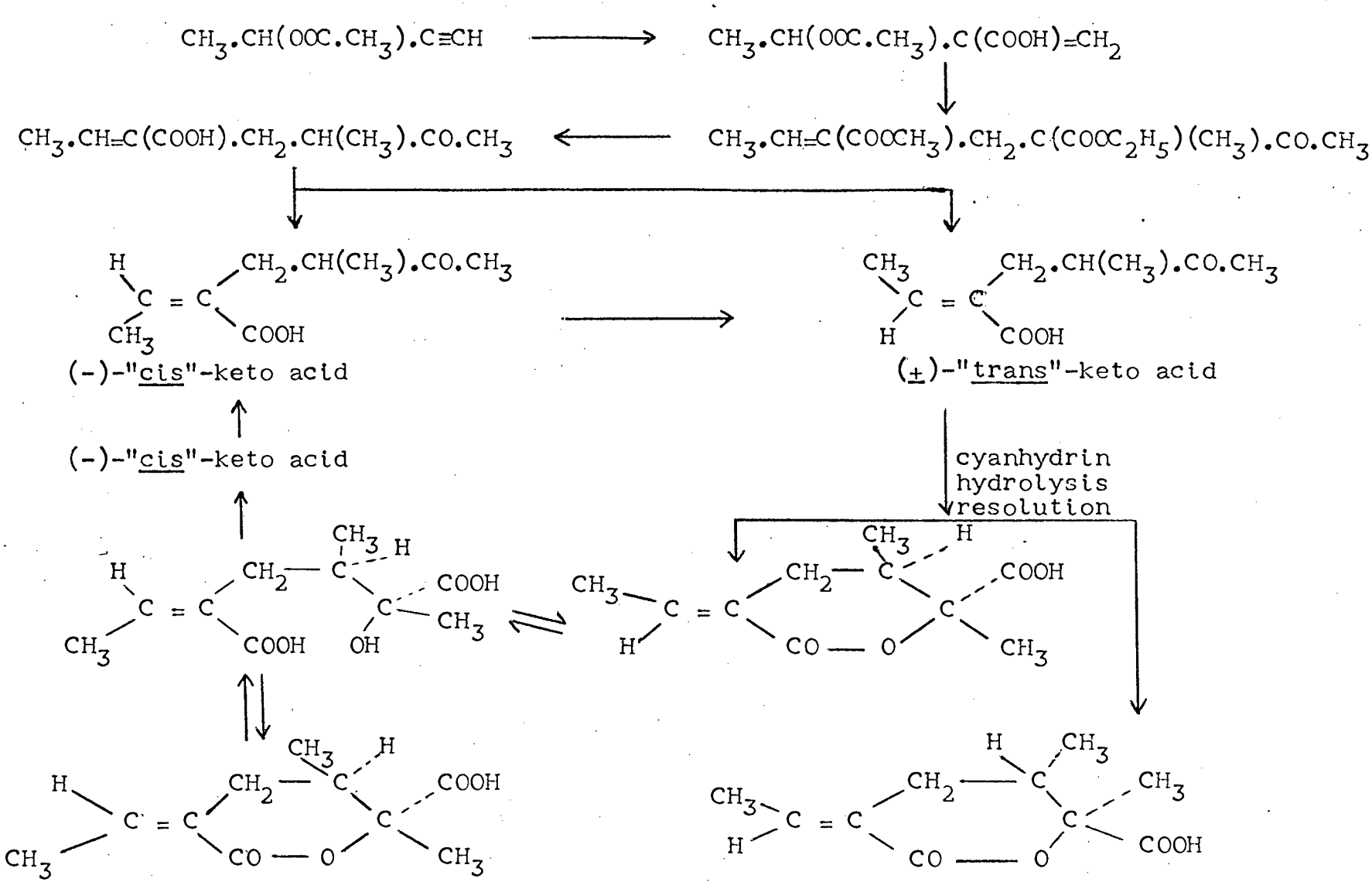


Chart I. The Culvenor-Geissman Synthesis & Interconversions of Senecic and Integerrinecic Acids.

Further studies of these "necic acids" have shown that in the U.V. spectra not only was $\lambda_{max.}$ for the cis form (215 mμ) less than that of the trans acid (218 mμ), but the $E_{max.}$ of the former was approximately half that of the latter.

Degradation of isatinecic acid and senecic acid to (+)-methyl-succinic acid[10] established the configuration of the asymmetric carbon $C_{(3)}$. The fact that lactone formation does not involve $C_{(3)}$ led Adams to conclude that this asymmetric carbon atom in retronecic and integerrinecic acids also remained unchanged. Further evidence of this was shown by the dilactone of hygrophyllinecic acid discovered by Richardson, Warren[11] and Schlosser[12] which has the same carbon skeleton as senecic acid and here again the C_3 is not affected during lactonization.

Usaramoensinecic acid from the Crotolaria alkaloid discovered by Adams and van Duuren[13] was found to be isomeric with senecic and integerrinecic acid and like senecic acid gave integerrinecic acid lactone. U.V. studies on the acid showed a maximum absorption at 215 mμ which was similar to that of senecic acid for which the cis configuration was assigned.

Since senecic, integerrinecic and usaramoensinecic acids give the same lactone, i. e. integerrinecic acid lactone, Adams concluded that the isomerism is not associated

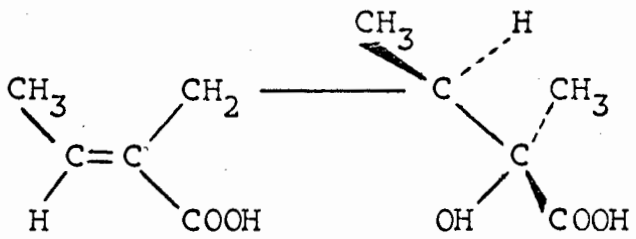
with the asymmetry at $C_{(3)}$ but more probably with the $C_{(2)}$. Hence Adams and co-workers showed that structure (II) (page 1.) can exist in three stereoisomeric forms which can be written showing the absolute configuration at $C_{(3)}$ (proved by formation of (+)-methyl-succinic acid).

Another acid found by Danilova and Konolova[3] called platynecic acid (obtained by alkaline hydrolysis of the alkaloid platyphylline) has shown to be isomeric with senecic acid and lactonized to integerrinecic acid lactone and has been clained to be the fourth member of this group.

Adams and co-workers[13] suggested that since senecic and usaramoensenecic acids are both cis and both undergo a change in configuration at the double bond to give the stable trans form of the lactone on treatment with the acid or alkali, it was suggested that the difference between the two acids must be due to the configuration at $C_{(2)}$.

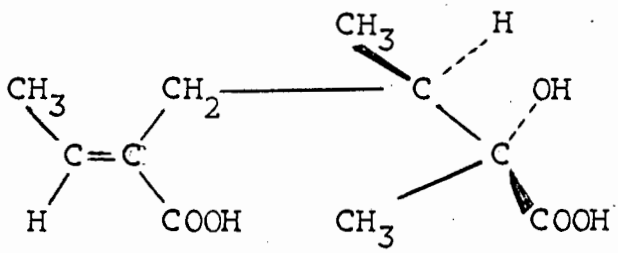
Furthermore Adams stated that in integerrinecic acid the $C_{(2)}$ will have its hydroxyl group cis to the lactonizing carboxyl since the acid and the lactone are interconvertable. In the other two acids the hydroxyl of the $C_{(2)}$ may be cis or trans with inversion taking place in one of these acids during lactonization. The author regards these terms cis and trans as very misleading and proposes to name the acids using the absolute configuration method according to Cahn, Ingold and Prelog[14].

The structure of the acids as given by Adams is shown in Chart II. Hence, the work of Adams and van Duuren seems to be the only mention of the asymmetry at $C_{(2)}$ since it was necessary to use it to explain the relationship of usaramoensenecic, senecic, and integerrinecic acid.



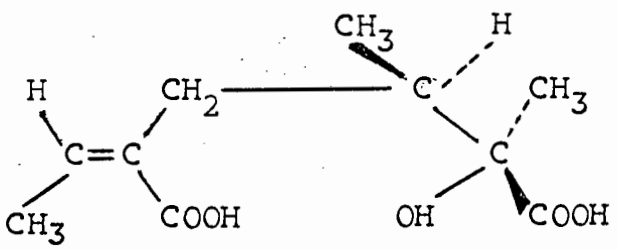
Old nomenclature : trans-cis-integerrinecic acid

New nomenclature : trans [2R,3R] integerrinecic acid *



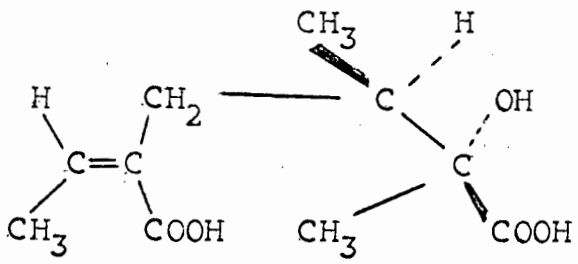
O.N. : trans,trans-platynecic acid

N.N. : trans [2S,3R] platynecic acid *



O.N. : cis,trans-senecic acid

N.N. : cis [2R,3R] senecic acid *



O.N. : cis,cis usaramoensenecic acid

N.N. : cis [2S,3R] usaramoensenecic acid *

Chart II. - The Structural Relationship of Integerrinecic, Platynecic and Usaramoensenecic acids.

* The term cis and trans refers to angelic and tiglic acid nomenclature and has been retained. It is acknowledged that the I.U.P.A.C. rules would dictate the interchange of these terms cis and trans.

DISCUSSION

DISCUSSION.

a) Present Studies.

No attempt has yet been made to confirm that the acids shown in Chart II differ at the $C_{(2)}$ centre. The author has set out to do this by converting isatinecic acid (cis) to senecic (cis) since the absolute configurations at $C_{(3)}$ in isatinecic acid and senecic acid are known to be the same.

The investigation had as its objective the conversion not only to senecic acid but also the synthesis and structure of usaramoensenecic and platynecic acid.

The conversion was performed, as shown in Chart III, by oxidation of dimethyl isatinecate (III) followed by a Grignard reaction on the resulting keto acid (IV) producing dimethyl sinecate and its isomers (V) and the final hydrolysis to yield a mixture of senecic acid and its isomers (VI).

The esters of the acid were used because the author wished to keep the reaction product in solution during the Grignard reaction since it was feared that the magnesium salt of the acid would be insoluble.

Although the Grignard addition should have been stereochemically directed there should be a small

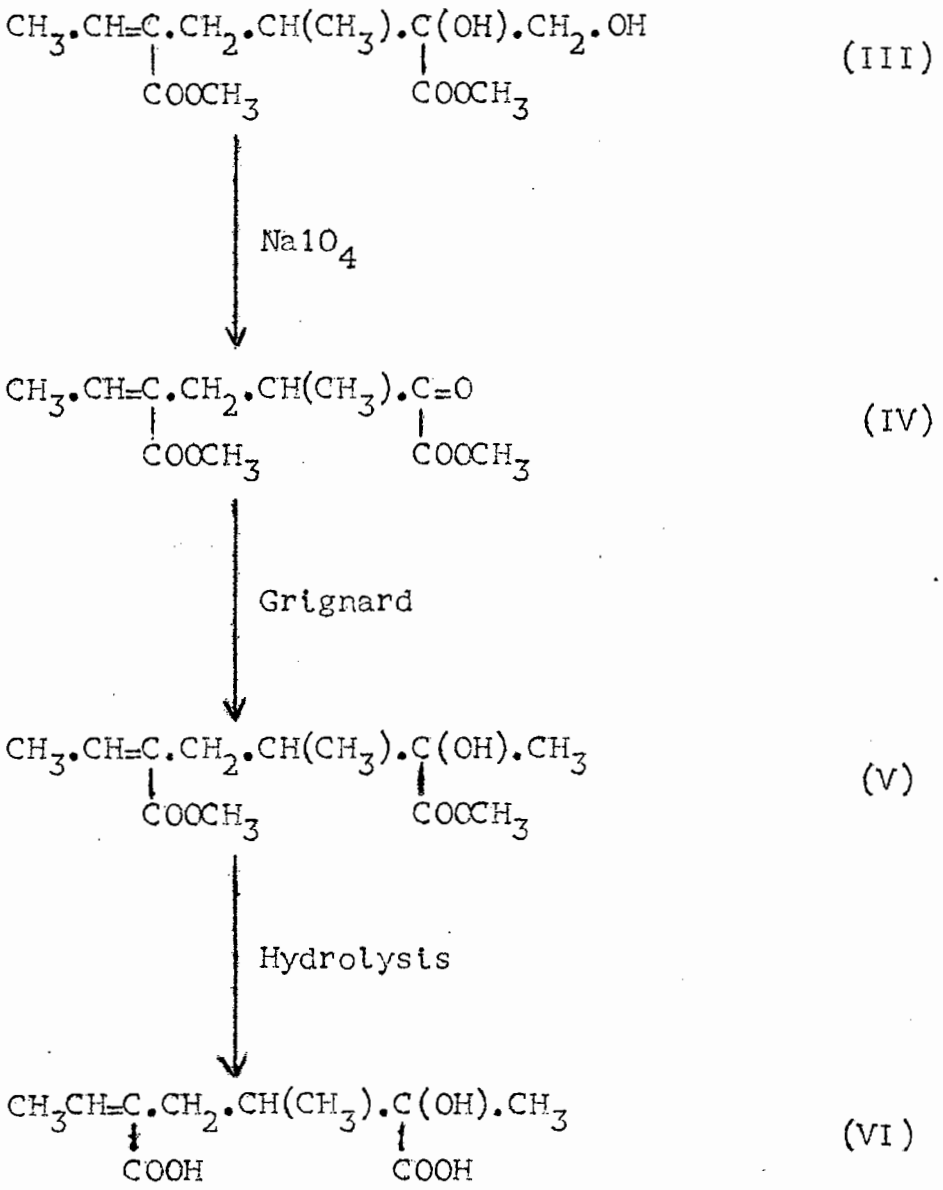


Chart III. - Conversion of Isatinecic Acid to Senecic Acid.

quantity of the second acid formed, that is its diastereoisomer.

The initial problem was to find a method which would give a good yield of dimethyl isatinecate, as shown in Chart IV, (VII; R = CH_3) since the known method according to Reimer and Downes[15], where a trans-esterification starting with retrorsine (VIII) is done directly on the alkaloid with methyl alcohol, only gives a 60% yield.

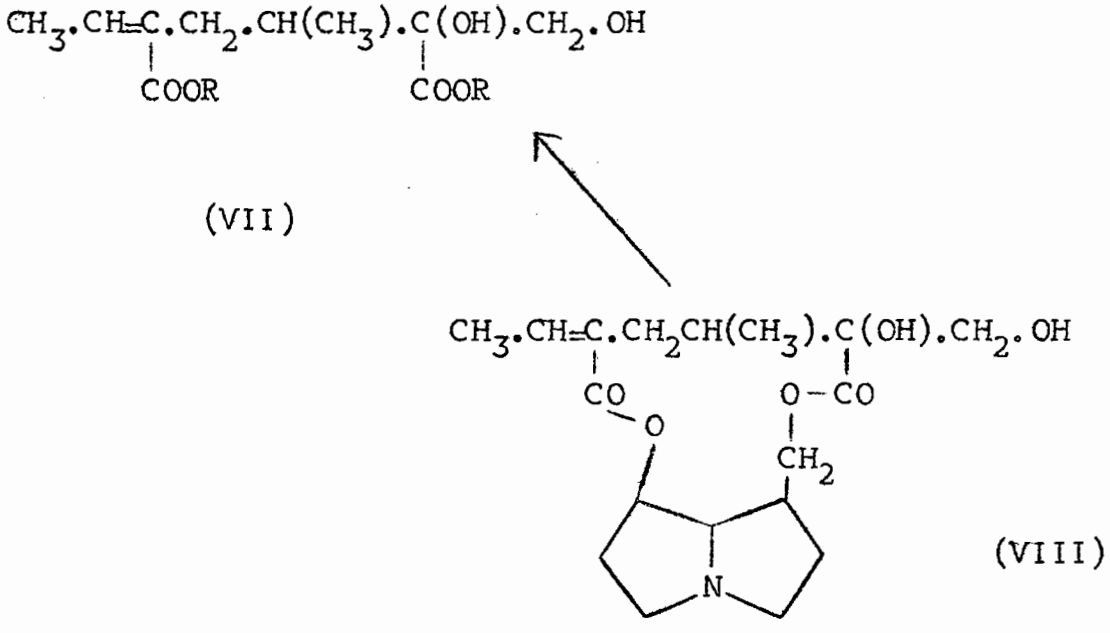


Chart IV. - The Preparation of Dimethyl Isatinecate.

Hence sodium isatinecate (VII; R = Na) was methylated with methyl iodide; but the yield was low approximating to 10%. Attempts to use silver isatinecate (VII; R = Ag), formed from barium isatinecate and silver nitrate, had to be carried out in the dark and methylation using methyl iodide gave an oily product which did not have a definite boiling point. In fact thin layer chromatography revealed a mixture of of six compounds, some of the latter probably being decomposition products brought about by silver oxide. The author eventually used the Reiner and Downes trans-esterification method: (VII) $\rightarrow$ (VII; R = CH_3).

The freshly prepared dimethyl isatinecate was treated with sodium periodate in an aliquot mixture of water and dioxan. This oxidation was instantaneous and quantitative; but the newly formed keto-ester had to be extracted within four minutes. Experiments showed that decomposition took place after four minutes and after eight minutes only a 70% yield could be obtained, but this remained constant even after three hours. The keto-ester was obtained as an optically active oil indicating that no racemisation had yet taken place.

The Grignard reaction which followed was performed using the apparatus shown on Fig. I, page 44

The Grignard reagent was first prepared and added to an etheral solution of keto-ester, in order to avoid excess of the Grignard reagent being present at any time during the reaction. This procedure was adopted to avoid excess reagent acting on the ester groupings.

Thin layer chromatography of the product produced four spots. The mixture was separated by column chromatography using neutral alumina and one of the four bands yielded a colourless optically inactive oil which was dimethyl senecate and its isomers.

Crystallisation from benzene yielded crystals which did not have a definite melting point and were optically inactive. The mixture of isomers could not be separated with thin layer or paper chromatography. With paper chromatography a variety of systems were used but all resulted in streaks being produced. Slow sublimation under reduced pressure effected no separation as shown by the infra-red spectrum: that of the sublimed product was identical with that of the residue as shown on appendix I, page 46.

The mixture was then oxidised with lead tetra-acetate to form a new oily optically inactive keto acid, as shown on Chart V. (X). This oxidation was first carried out by Warren and Kropman[16] on senecic

acid and later by Culvenor and Geissman[4] during their synthesis of senecic and integerrinecic acids.

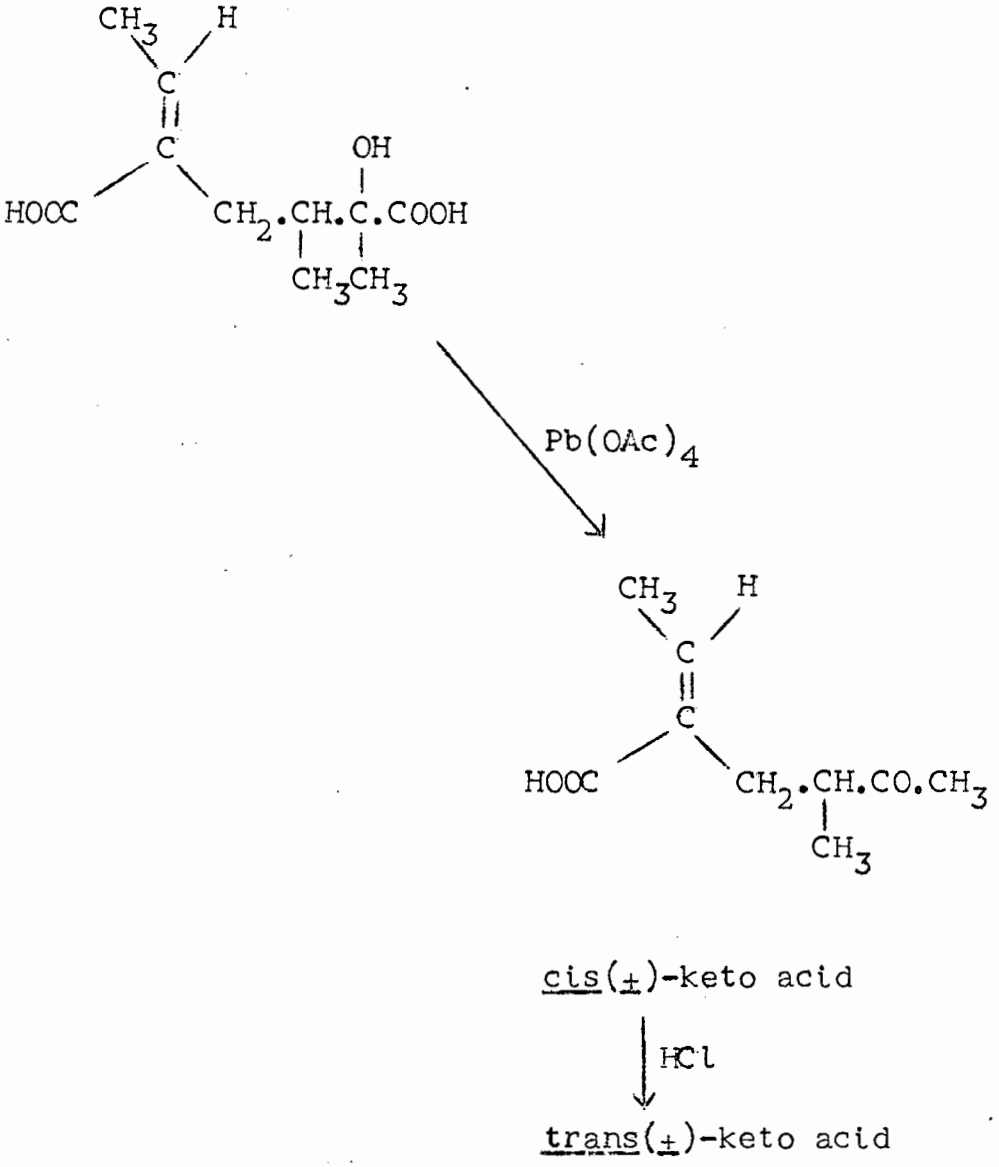
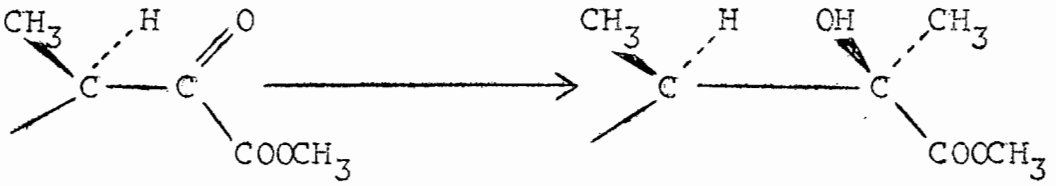


Chart V. - The Lead Tetra-acetate Oxidation of Senecic Acid.

The 2,4-dinitrophenylhydrazine derivative of this compound had a lower melting point than that quoted by Culvenor and Geissman[4]. This led the author to suspect that some of the trans form of the keto acid might have been present. To test this assumption the keto acid was then converted to the trans form by treating it with concentrated hydrochloric acid when crystals of the trans keto acid, m. p. 47 - 50°, were obtained whose 2,4-dinitrophenylhydrazine derivative, m. p. 145°, had the same melting point as the trans derivative, m. p. 145°, reported by Culvenor and Geissman[4]. Hence the original possibility was confirmed, namely that a mixture of cis and trans dl and d'l' of the acids had been obtained during conversion.

These results clearly demonstrated that Cram's rule[17] cannot be applied directly to predict the configuration of the resulting product because had Cram's rule been applicable then, as shown on Chart VI, oxidised dimethyl isatinecate (XI) should have given (+)-dimethyl senecate (XII) on reacting with methyl magnesium iodide.



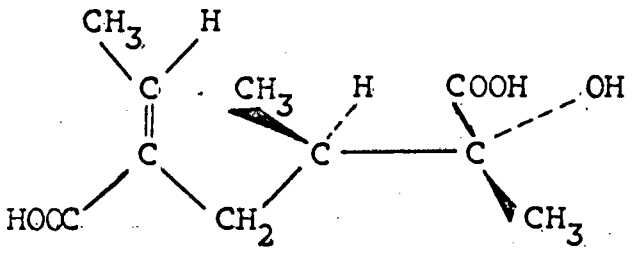
(XI) (XII)

Chart VI. - Grignard Reaction on Dimethyl Isatinecate

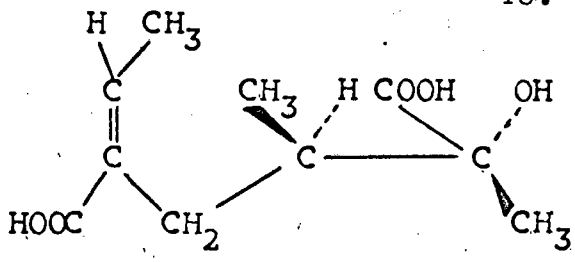
It is also of interest that the oxidation of senecic acid (XIIIa) carried out by Schlosser[18], as shown on Chart VII, to the keto acid (XIV) and the addition of hydrogen cyanide to give (XVI) followed by hydrolysis gave integerrinecic acid (XIIIb) which is contrary to Cram's rule which would predict (XV).

It is difficult to understand the stereospecific addition to (XIV) not following Cram' rule. The addition of the Grignard reagent, however, to oxidised dimethyl isatinecate (XI) gave a mixture of products which could well have been resulted from racemisation and the more random addition of the carbonyl carbon.

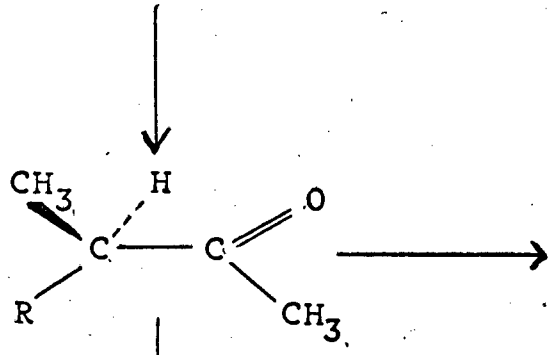
It is of significance that for his investigations Cram[17] deliberately chose a system containing no groups on $C_{(1)}$ capable of giving complexes with the reagent involved in the production of asymmetry at $C_{(2)}$. In the presence of such a group, e. g., in the aluminium alkoxide reduction of $Ar.CO(NHR).CH_2.OH$, the steric



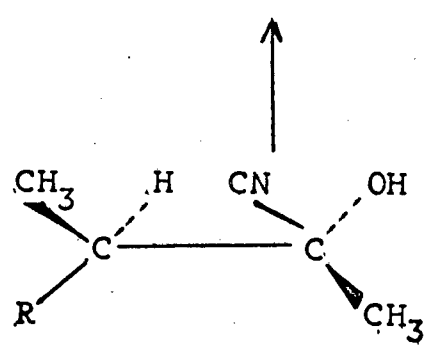
Senecic acid - (XIIIa)



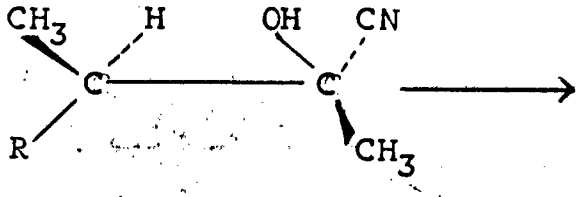
Integerrinecic acid - (XIIIb)



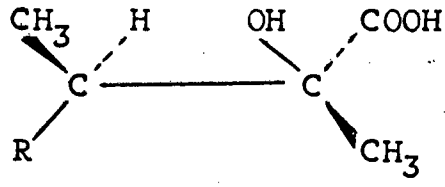
(XIV)



(XVI)



(XIVa)

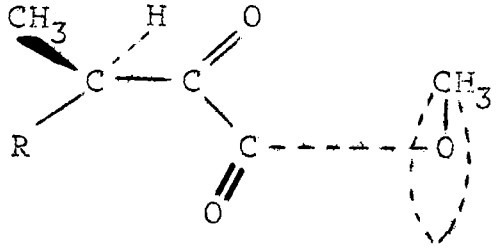


(XV)

Chart VII. - Possible modes of addition to the methyl-ketone ester obtained from the oxidation of dimethyl senecate: according to Cram's Rule, (XIV) ⟶ (XIVa) ⟶ (XV); according to experiment, (XIV) ⟶ (XVI) ⟶ (XIIIb).

course of the reaction appears to be determined by the free terminal hydroxyl group.

In the example under discussion we undoubtedly have the keto group flanked on both sides with steric controlling groups, as shown on (XVII).



(XVII)

Another attempt was carried out, this time on the acid alone, with the hope that the absence of the methyl ester groups would produce a more significant result.

Retronecic acid, obtained by the alcoholic potassium hydroxide hydrolysis[19] method from retrorsine was was oxidised with sodium periodate to yield the (+)-keto acid as an oily product. The reaction was carried out in acidic conditions since racemisation took place within minutes at pH 12 or 13 to produce an optically inactive mixture. At pH 1 no racemisation took place. The (+)-keto acid

was reacted with methyl-magnesium iodide to produce a gum, which after a considerable amount of difficulty formed fine white needle like crystals of integerrinecic acid. The infra-red spectrum of these crystals was identical with that of integerrinecic acid from senecic acid lactone, as shown in Appendix II, page 47.

The mother liquors, from which the integerrinecic acid had been separated, could not be induced to crystallize and remained as a clear oil, analysing to that expected for the Grignard reaction product, $C_{10}H_{16}O_5$.

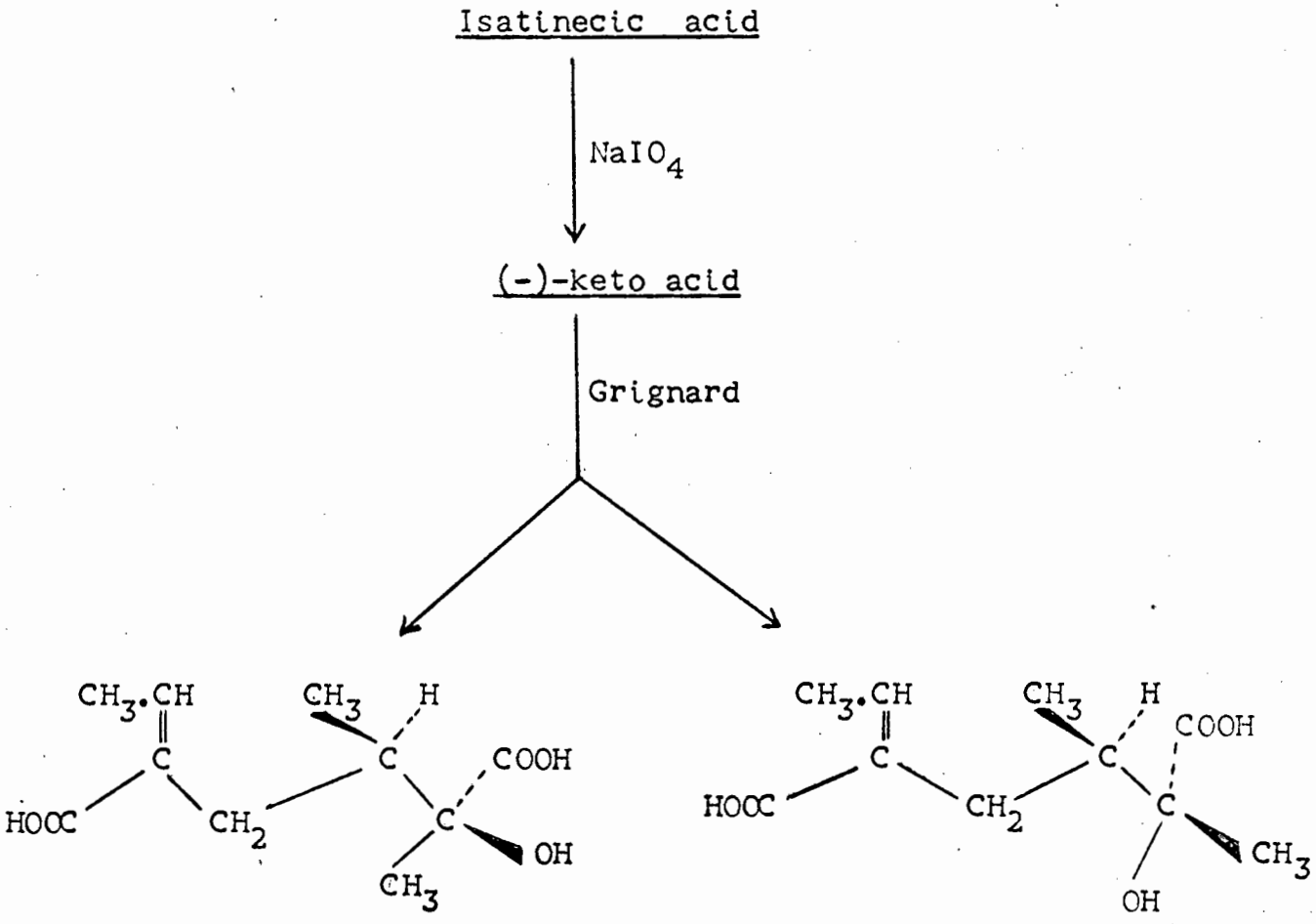
The investigation on the acids reported above parallels work carried out by Mattocks[20] in these laboratories: the oxidation of retrorsine to the cyclic keto-ester and the Grignard reaction on this product. The resulting compound was either the expected senecionine or isosenecionine according to the experimental conditions. Senecionine and isosenecionine were shown to be the retronecine esters of (+)-senecic and (-)-senecic acids.

The yields obtained by Mattocks were small and a considerable amount of material remained unresolved. In view of the experiments with the acids themselves

it was envisaged that the residual material might be a source of the platynecic and/or usaramoensenecic acids. Hence the author repeated the above experiments and it was observed that during the Grignard reaction a yellowish insoluble resin was also formed. The gum obtained at the end of the experiment was dissolved in some ethyl acetate and passed through a column. Senecione was obtained but no other alkaloid. Hence the development of the resin was responsible for Mattock's low yield and not, as was hoped, the formation of new alkaloids.

While the above experiments were in progress Mattocks[21] reported that he had extended his studies, started in these laboratories, (see below) to include the oxidation and Grignard reaction on isatinecic acid. He oxidised isatinecic acid with sodium metaperiodate, under acidic conditions, to form the (-)-keto acid. This was followed by a Grignard reaction and rather unexpectedly the (+) diastereoisomer (XIX) of senecic acid (XVIII) was obtained as the main product, as shown on Chart IX. The new diastereoisomer has been called diasenecic acid. (see Appendix III, page 48).

Diasenecic acid was oxidised with lead tetra-acetate and it gave the same keto acid (X) as the



Senecic acid.

(XVIII)

Diasenecic acid.

(XIX)

Chart IX. - The Formation of Senecic and Diasenecic Acids.

one obtained by a similar oxidation on senecic acid (see Chart V). Hence the two acids have the same configuration at $C_{(3)}$. Furthermore, U. V. studies performed by the author on diasenecic acid showed that the latter has a cis configuration which is the same as senecic acid. This means that the senecic and diasenecic acids differ only at $C_{(2)}$ as shown by formulae (XVIII) and (XIX) on Chart IX.

Racemic diasenecic acid together with senecic acid has also been recently synthesized by Edwards et al[22].

b) Conclusion.

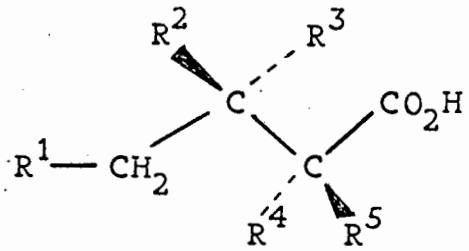
As has been shown above the properties of diasenecic acid do not correspond to those of usaramoensenecic acid. Thus, the latter cannot be a stereoisomer of senecic acid. The author is convinced that usaramoensenecic acid does not exist at all. Furthermore, Culvenor and Smith[23] recently carried out a re-investigation on the alkaloids of C. usaramoensis. This plant was found to contain usaramine together with integerrimine, senecionine and retrorsine, but no usaramoensine. They suggest that the latter was a mixture of integerrimine, senecionine, and usaramine. Retronecic acid (from usaramine) would

account for the high m. p. of "usaramoensenecic acid".

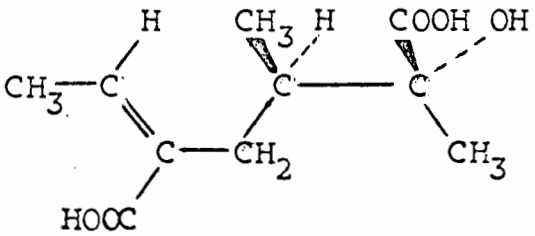
Finally, platynecic acid has not been found in any of the syntheses and the only acid which would fit its stereo-chemical structure is trans-(+)-diasenecic acid. Furthermore, it would appear, as suggested by Leonard[24], that this acid could be a mixture of integerrinecic acid and senecic acid rather than a pure chemical individual. This would explain more satisfactorily the finding that platynecic acid and senecic acid, after absorption of two atoms of hydrogen catalytically, are convertible to the same saturated lactonic acid as obtained from integerrinecic acid lactone by catalytic hydrogenation. Platynecic acid is probably senecic acid in the alkaloid platyphylline.

The isomers of senecic acid are now all known as shown on page 25.

Isomers of Senecic Acid.

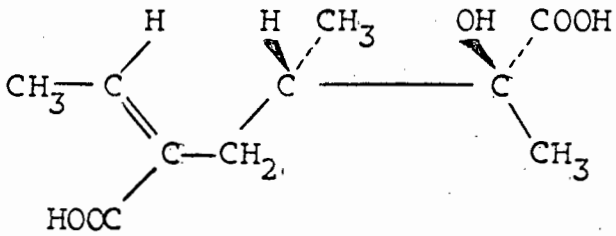


ACID	R^1 / $MeCH{=}C(CO_2H)$	R^2	R^3	R^4	R^5	NO
(+) senecic	cis	Me	H	OH	Me	(XX)
(-) senecic	cis	H	Me	Me	OH	(XXI)
(+) integerrinecic	trans	Me	H	OH	Me	(XXII)
(-) integerrinecic	trans	H	Me	Me	OH	(XXII)
(+) diasenecic	cis	Me	H	Me	OH	(XXIV)
(-) diasenecic	cis	H	Me	OH	Me	(XXV)
(+) trans-diasenecic	trans	Me	H	Me	OH	(XXVI)
(-) trans-diasenecic	trans	H	Me	OH	Me	(XXVII)



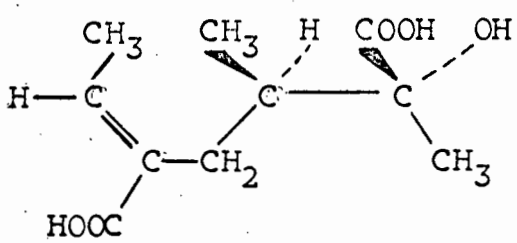
(XX)

(+)-Senecic acid



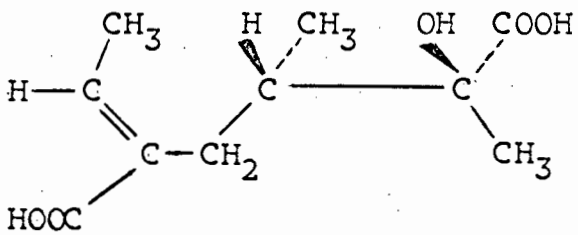
(XXI)

(-)-Senecic acid



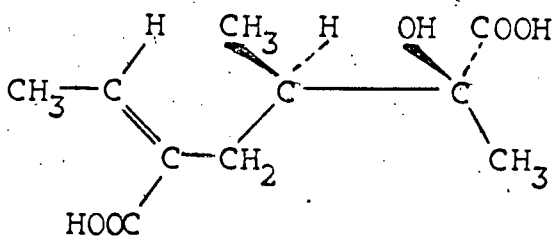
(XXII)

(+)-Integerrinecic acid



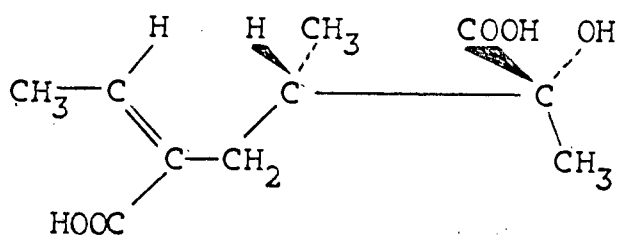
(XXIII)

(-)-Integerrinecic acid



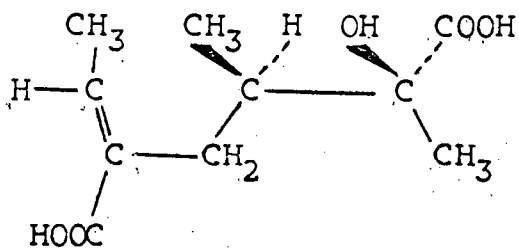
(XXIV)

(+)-Diasenecic acid



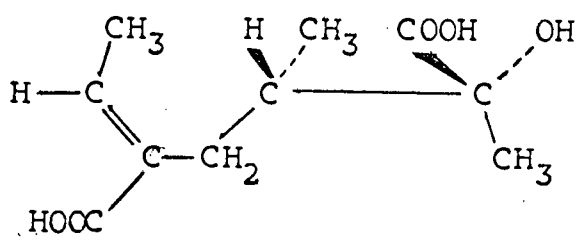
(XXV)

(-)-Diasenecic acid



(XXVI)

(+)-<u>trans</u>-Diasenecic acid



(XXVII)

(-)-<u>trans</u>-Diasenecic acid

Chart X. - The Absolute Configuration of Senecic Acid and its Isomers.

EXPERIMENTAL.

to give retrorsine, m. p. 216 - 217°. Richardson and Warren give m. p. 217°. Yield: 20 g.

2. HYDROLYSIS OF RETRORSINE

Retrorsine (10 g.) and hydrated barium hydroxide ($Ba(OH)_2.8H_2O$) dissolved in water (250 ml.) were refluxed for 3 - 4 hours. Carbon dioxide was then passed into the solution until all the barium carbonate was precipitated. After filtration the solution was evaporated to dryness, and the solid continuously extracted with ethanol (5 hours) in order to remove the base. The remaining solid was re-crystallised from water to give barium isatinecate as a crystalline powder.

3. PREPARATION OF SODIUM ISATINECATE FROM BARIUM ISATINECATE

To a solution of barium isatinecate (8 g.), sodium sulphate solution was added in excess. Sodium isatinecate was formed and barium sulphate was precipitated. After filtration the solution was evaporated to dryness under reduced pressure to give crystalline sodium isatinecate. Yield: 5 g.

4. METHYLATION OF SODIUM ISATINECATE WITH METHYL IODIDE

Sodium isatinecate (1 g.) and methyl iodide (1.6 g.)

in absolute methanol (2 ml.) were heated for one hour in a sealed tube in a water bath. The solution was cooled, water added and extracted with ether. The etheral layer was dried over anhydrous sodium sulphate and the ether was evaporated off, leaving a small quantity of oily substance which was distilled at 125°/ 0.01 mm. to give methyl isatinecate as an oil. Yield: 0.1 g.

Found: C, 55.1; H, 7.9%.

Calculated for $C_{12}H_{20}O_6$: C, 55.4; H, 7.8%.

5. PREPARATION OF DIMETHYL ISATINECATE USING SILVER ISATINECATE AND METHYL IODIDE

Silver nitrate (20 g.) was added to a solution of barium isatinecate (20 g.), the whole was heated and stirred on a water bath for 15 minutes in the dark. The precipitated silver isatinecate was filtered and dried under reduced pressure. It was then refluxed with methyl alcohol (10 ml.) and methyl iodide (13 g.) for three hours during which a white precipitate of silver iodide was formed. After filtration the precipitate was washed with methanol. The filtrate was evaporated to yield an oily substance, which on distillation gave an oily product at 125 - 130°/ 0.01 mm.

Found: C, 54.5; H, 7.6%.

Calculated for $C_{12}H_{20}O_6$: C, 55.4; H, 7.8%.

Thin layer chromatography using ethyl alcohol as a developer on silica plates revealed six spots thus indicating a mixture.

6. TRANS-ESTERIFICATION OF RETRORSINE USING METHYL ALCOHOL

The esterification was carried out following the Reimer Downes[15] method at room temperature, with a modification as to the length of time taken over the shaking and refluxing of certain stages of the method. Retrorsine (6 g.) in absolute methanol (60 ml.) together with potassium metal (0.08 g.) was shaken for thirty minutes then refluxed for three to four hours. Methanolic hydrochloric acid was added until the solution was neutral. The solution was filtered and approximately four fifths of the methanol removed under pressure. Iced water (15 ml.) was added and the resultant mixture was thoroughly shaken out with ether. (4 x 75 ml.). The ether, dried over anhydrous sodium sulphate and filtered, gave dimethyl isatinecate as an oil b. p. 135 - 136°/ 0.01 mm.

Found: C, 55.1; H, 7.9%.

Calculated for $C_{12}H_{20}O_6$: C, 55.4; H, 7.8%.

7. OXIDATION OF DIMETHYL ISATINECATE USING SODIUM PERIODATE

Dimethyl isatinecate (2 g.) was dissolved in a mixture of dioxan (12 ml.) and water (4 ml.) at room temperature. 0.3M sodium periodate (25.8 ml.) was added and after two minutes water (20 ml.) was added immediately, the new ketone formed was extracted with ether four times. The ether together with smaller quantities of dioxan and water were distilled off under reduced pressure to give dimethyl 2-methyl-hex-3-ene-1-one-1,4-dicarboxylate as an oily liquid at 105 - 108°/ 0.01 mm. Yield: 1.2 g.

Found: C, 57.1; H, 7.2%.

Calculated for $C_{11}H_{16}O_5$: C, 57.9; H, 7.1%.

$[\alpha]_D^{20}$ +3.55°. Therefore it was not racemised.

Addition of 2,4-dinitrophenylhydrazine to the oil in ethanol formed a 2,4-dinitrophenylhydrazone derivative of the keto ester as a dark brown oil which could not be crystallised.

Found: C, 49.6; H, 5.6; N, 13.8%.

Calculated for $C_{17}H_{20}N_4O_8$: C, 50.0; H, 5.0; N, 13.1%.

8. REACTION OF OXIDISED DIMETHYL ISATINECATE WITH METHYL-MAGNESIUM IODIDE

The reaction was carried out using the apparatus shown on page 44. The Grignard reagent was first prepared.

Methyl iodide (1.7 ml.; 2.5 moles) was slowly added to clean dry magnesium ribbon (0.7 g.; 2.5 atoms) in distilled dry ether (20 ml.) at room temperature and after the addition was completed the mixture was warmed on a water bath. When all the magnesium ribbon had been used up, the resultant mixture was added drop by drop to a mixture of the keto ester (6.4 g.; 1 mole) in dry ether (20 ml.). This reaction was kept below 10^{o}. The new complex was decomposed by the addition of cold water (40 ml.) with a little dilute hydrochloric acid. The ether layer separated, and the aqueous layer was shaken with four lots of ether. The combined ether layers were dried over anhydrous sodium sulphate, and shaken with a little sodium thiosulphate to remove any iodine present. Evaporation of the ether produced an oil which distilled at 115 - 125^{o}/ 0.01 mm. Thin layer plates of alumina with benzene and ethyl alcohol (96:4) mixture as a developer revealed four spots.

9. CHROMATOGRAPHY OF THE ABOVE MIXTURE

A 3 cm. x 27 cm. column was prepared from neutral aluminium oxide (150 g.) ("CAMAG" M.F.C.) suspended in dry benzene. To this was added the oily mixture (6 g.) dissolved in a few drops of benzene, and a flow of 150 ml./ hour of benzene was maintained using air

pressure. Fraction (40 ml.) were collected, and after 560 ml. of benzene had passed ethyl acetate (100 ml.) was introduced and finally ethyl alcohol (100 ml.). Four bands were formed on the column.

The results obtained were as follows:-

Fraction	ml.	Material
1 - 4	160	-
5	40	First band
6	40	-
7	40	Part of second band
8	40	Rest of second band
9 - 10	80	-
11 - 16	240	Third band
17	40	Part of fourth band
18 - 20	80	Rest of fourth band
20	40	-

Fraction 5 on evaporation yielded a few milligrams of brown oil which was discarded.

Fraction 7-8 on evaporation yielded a clear oil.

Found: C, 64.3; H, 8.9%.

Calculated for $C_{12}H_{20}O_5$: C, 59.0; H, 8.3%.

Fractions 11-16 on evaporation yielded dimethyl sinecate as a clear oil. $[\alpha]_D^{20} \pm 0^\circ$. Yield: 5 g.

Found: C, 59.0; H, 8.5%.

Calculated for $C_{12}H_{20}O_5$: C, 59.0; H, 8.3%.

Fractions 17-19 on evaporation yielded an oil.

Found: C, 56.5; H, 8.4%.

Calculated for $C_{12}H_{20}O_5$: C, 59.0; H, 8.3%.

10. PREPARATION OF BARIUM SENECATE FROM DIMETHYL SENECATE

Dimethyl senecate (1.0 g.) was dissolved in methanol (8 ml.) and was added to a solution of barium hydroxide (2.8 g.) and it was refluxed for eight hours. Carbon dioxide was passed into the hot solution and the barium carbonate precipitated was filtered off. The solution was evaporated to dryness and barium senecate was obtained. Yield: 0.91 g.

11. HYDROLYSIS OF BARIUM SENECATE

Barium senecate (0.91 g.) was dissolved in water (10 ml.) and passed through an ion exchange column (Regenerated Amberlite Resin - I.R. - 120; column, 1.3 cm. x 4.0 cm.). The aqueous solution was extracted with ether five times. The ether was dried over anhydrous sodium sulphate and evaporated to produce a gum. Crystallisation from benzene yielded white crystals of senecic acid and

isomers, m. p. 135 - 147°. $[\alpha]_D^{20}$ 0° (in ethanol)

Found: C, 55.6; H, 7.4%.

Calculated for $C_{10}H_{16}O_5$: C, 55.5; H, 7.5%.

12. SUBLIMATION OF SENECIC ACID AND ISOMERS

The mixture of senecic acid and its isomers was sublimed at 110°/ 0.001 mm., for four hours. A gummy deposit was obtained on the cold finger which was taken up with benzene and the solution concentrated to yield crystals, m. p. 135 - 147°.

Found: C, 55.3; H, 7.5%.

Calculated for $C_{10}H_{16}O_5$: C, 55.5; H, 7.5%.

The infra-red spectrum of this compound was the same as the infra-red spectrum of the residue, as shown in Appendix I, page 46.

13. OXIDATION OF SENECIC ACID AND ISOMERS WITH LEAD TETRA-ACETATE

Lead tetra-acetate (1.2 g.) was added to a solution of senecic acid (0.5 g.) in dry benzene (100 ml.). The solution was warmed to 50°, and shaken for one hour. Excess oxidant was destroyed with ethylene glycol (2 drops) the solution filtered and the benzene evaporated off. The residue was dissolved in hot water, and diluted

sulphuric acid was added. After removing the lead sulphate formed by filtration, the solution was extracted four times with ether. The ether dried over anhydrous sodium sulphate was evaporated to yield (±)-5-methyl-2-hepten-6-one-3-carboxylic acid. $[\alpha]_D^{20}$ 0° (in absolute alcohol).

Found: C, 64.2; H, 8.7%.

Calculated for $C_9H_{14}O_3$: C, 63.5; H, 8.3%.

The (±)-keto acid formed a 2,4-dinitrophenylhydrazone derivative, m. p. 160 - 161°., from ethanol as brown irregular crystals. Culvenor and Geissman[4] quote m. p. 189 - 190°. for the 2,4-dinitrophenylhydrazone derivative of (±)-cis-keto acid. The (±)-keto acid was heated for thirty minutes at 100° in concentrated hydrochloric acid and recovered with ether after the addition of water. The product was taken up in pentane cooled to 0° and needles of (±)-trans-keto acid m. p. 49 - 50° were obtained. The 2,4-dinitrophenylhydrazone crystals obtained from ethanol melted at 135 - 137°. Purification of the crystals by passing them through a small column of neutral aluminium oxide suspended in ethanol yielded brown crystals, m. p. 144 - 146°. (Culvenor and Geissman quote m. p. 145 - 146°).

14. ALCOHOLIC POTASSIUM HYDROXIDE HYDROLYSIS OF RETRORSINE

To retrorsine (10 g.) dissolved in ethanol (80 ml.) was added solid potassium hydroxide (4 g.; 1.3 mol.) and then boiled under reflux. Within five minutes crystals separated and after ten minutes the content of the flask was one mass of crystals. The crystals were filtered off, thoroughly washed with ethanol and dried to yield potassium retronecate.

15. ISOLATION OF THE ACID

The potassium retronecate was dissolved in cold water (20 ml.), the solution neutralised with 8N sulphuric acid until just acid to congo red, and filtered. The filtrate on evaporation gave crystals, which, washed with water, yielded crystals of retronecic acid, m. p. 181°.

16. OXIDATION OF RETRONECIC ACID

A solution of sodium periodate (2.0 g.) in water (10 ml.) was added to a solution of retronecic acid (2.0 g.) in water (10 ml.), at room temperature. A few drops of dilute hydrochloric acid were added to bring the pH to one. After two minutes the solution was extracted with six lots of ether. The combined etheral extracts were

dried over anhydrous sodium sulphate and the ether was evaporated off to yield the keto acid as an oily product (1.85 g.), $[\alpha]_D^{19}$ +10.2° (in ethanol).

Found: C, 53.4; H, 7.1%.

Calculated for $C_9H_{12}O_5$: C, 53.9; H, 6.0%.

The 2,4-dinitrophenylhydrazine derivative of the keto acid formed from ethanol red brown needles, m. p. 185°.

Found: C, 42.7; H, 4.9; N, 12.4%.

Calculated for $C_{15}H_{16}N_4O_8$: C, 43.3; H, 4.2; N, 14.0%.

17. REACTION OF OXIDISED RETRONECIC ACID WITH METHYL-MAGNESIUM IODIDE

The reaction was performed using the apparatus as shown on page 44. The Grignard reagent was first prepared.

Methyl iodide (1.7 g.; 2.5 moles) was slowly added to clean magnesium ribbon (0.3 g.; 2.5 moles) in distilled dry ether (15 ml.) at room temperature, at first, and later the mixture was warmed on a warm bath. When all the magnesium ribbon was used up, the resultant mixture was added drop by drop to the keto acid (1.0 g.; 1 mole) in dry ether (10 ml.). This reaction was kept below 10°. On completion, the complex formed, was decomposed by the addition of cold water (30 ml.) and diluted hydrochloric acid (2 - 3 ml.). The ether layer

separated out, and the aqueous layer was shaken with four lots of ether. The combined ether layers were shaken with a little sodium sulphite to remove any iodine present and were then dried over anhydrous sodium sulphate. The ether was evaporated off to yield a light brown gum. After various attempts to crystallise the gum failed a successful crystallisation was obtained by washing the gum with ethyl acetate and evaporating off the ethyl acetate extracts. These also yielded a gum which was readily soluble in a few drops of benzene. This solution was added to light petroleum (b. p. 60 - 80°) (10 ml.) and white needle like crystals of integerrinecic acid were obtained m. p. 148° (Kropman and Warren[7] quote 151°). $[\alpha]_D^{20}$ +15.8° (in ethanol).

Found: C, 54.6; H, 7.5%.

Calculated for $C_{10}H_{16}O_5$: C, 55.5; H, 7.5%.

The infra-red spectrum was identical as that of integerrinecic acid formed from senecic acid lactone, as shown on Appendix II, page 47.

Mixed melting point with integerrinecic acid was 150°.

Attempts to crystallise the residual gum with several solvents failed.

Found: C, 54.4; H, 7.2%.

Calculated for $C_{10}H_{16}O_5$: C, 55.5; H, 7.5%.

18. OXIDATION OF RETRORSINE

A solution of retrorsine (4 g.) in dilute hydrochloric acid (20 ml.) was neutralized with sodium hydroxide and mixed with sodium metaperiodate (4 g.) in water (24 ml.) at room temperature. After one minute excess periodate was destroyed by adding ethylene glycol (1.2 ml.). 2N hydrochloric acid (8 ml.) was added and the solution was kept, at room temperature, for three hours. The pH was then adjusted to eight with a saturated solution of disodium hydrogen phosphate, and immediately extracted with four lots of chloroform. The combined extracts were dried for a few minutes and the chloroform was removed at room temperature under reduced pressure, to yield an oily residue which crystallised on rubbing with a few drops of ether. Yield 3 g.

19. REACTION OF CYCLIC KETO ESTERS WITH METHYL-MAGNESIUM IODIDE

The reaction was carried out using the apparatus shown on page 44. The Grignard reagent was first prepared.

Methyl iodide (2.0 ml.; 3 moles) was slowly added to clean dry magnesium ribbon (0.68 g.; 3 moles)

in distilled dry ether (20 ml.) at room temperature, and after the addition was completed the mixture was warmed on a water bath. When all the magnesium ribbon had been used up, the resultant mixture was added drop by drop to a mixture of the cyclic keto ester (3 g.; 1 mole) in dry ether (20 ml.). During the course of the reaction which was kept well below 10^{o}, a yellow resinous deposit was also formed. The new complex was decomposed by the addition of cold water (40 ml.) with a little dilute hydrochloric acid. The ether layer which separated was washed with a little dilute hydrochloric acid (5 ml.). and the combined aqueous layers were washed with ether, basified with ammonia and extracted with six lots of chloroform. The combined extracts were dried and concentrated under reduced pressure to yield a gum. Yield 1 g.

20. CHROMATOGRAPHY

A 1 cm. x 18 cm. column was prepared from neutral aluminium oxide (25 g.)("CAMAG" M.F.C.) suspended in ethyl acetate. To this was added the gum (1 g.) dissolved in ethyl acetate (2 ml.) and a flow of 150 ml./ hour of ethyl acetate was maintained. Fractions (8 ml.) were collected, and after 104 ml. of ethyl acetate had passed, ethyl alcohol (50 ml.)

was introduced and finally water (50 ml.)

The results obtained were as follows:-

Fraction	ml.	Material
1 - 2	16	-
3	8	Part of first band
4	8	Rest of first band
5 - 7	24	-
8	8	Second band
9 - 13	40	-
14	8	Part of third band
15	8	Rest of third band
16 - 25	108	-

Fractions 3-4 on evaporation yielded a few milligrams of brown oil.

Fraction 8 on evaporation yielded a couple of milligrams of brownish oil.

Fractions 14-15 on evaporation yielded a gum, which gave crystals of senecionine, on adding a few drops of ethyl acetate, m. p. 230 (decomp) $[\alpha]_D^{20}$ -50.3° (in ethanol). The infra-red spectrum of this compound was similar to the spectrum of senecionine (see Appendix V, page 50).

Found: C, 62.1; H, 8.3; N, 3.9%.

Calculated for $C_{18}H_{25}O_5N$: C, 64.5; H, 7.5; N, 4.2%.

21. THE GRIGNARD APPARATUS

The Grignard reactions were carried out using the apparatus shown on Fig.I, page 45. The Grignard reagent was first prepared in flask (D) having first closed taps (A) and (C). Tap (A) was opened until a complete vacuum was obtained in section (B). Tap (A) was closed and tap (C) was opened. The Grignard reagent passed into section (B) and tap (C) was closed. Section (B) was then detached from flask (D) and attached to flask (E) which contained a solution of the ketone in dry ether. The Grignard solution from section (B) was then passed into flask (E) drop by drop.

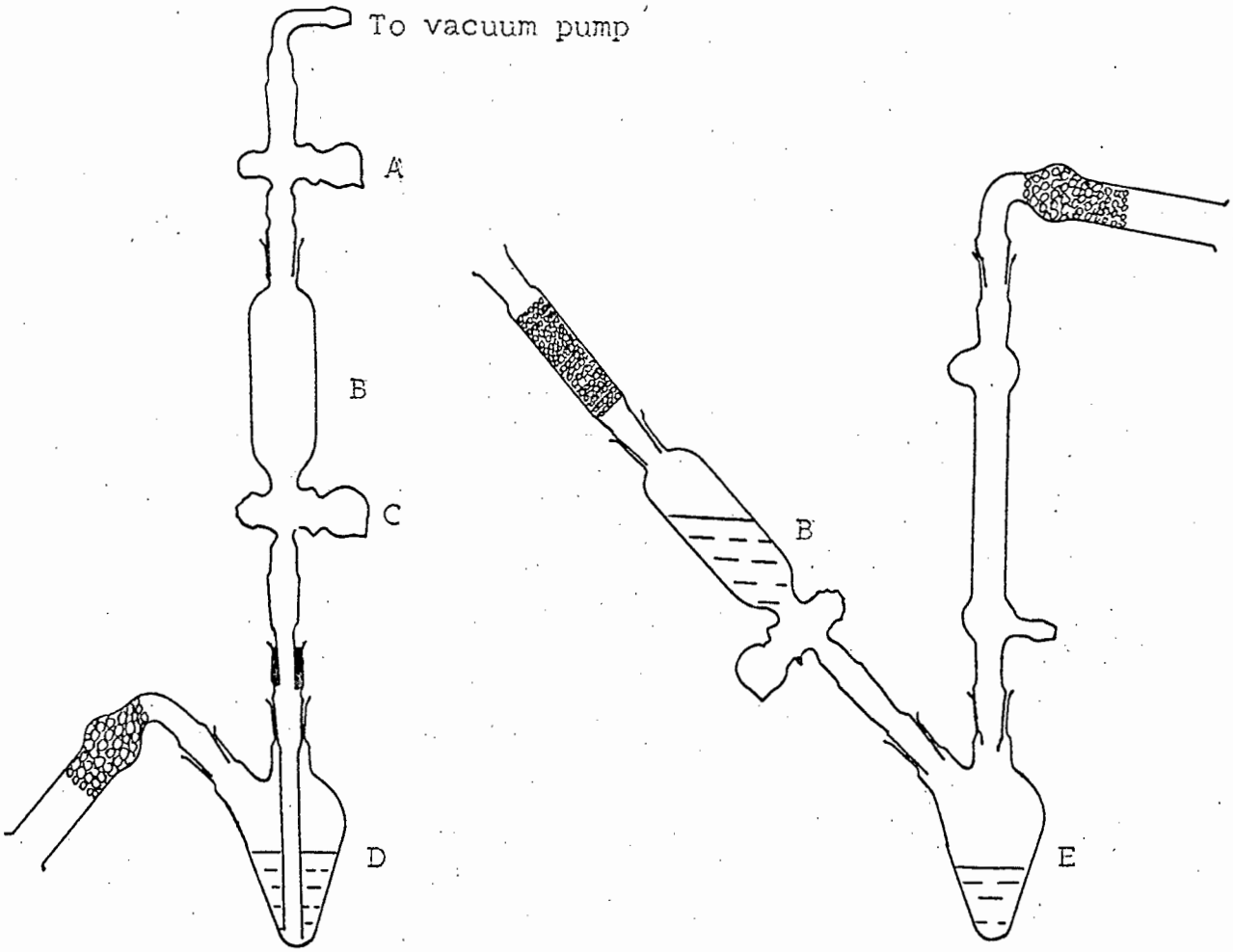
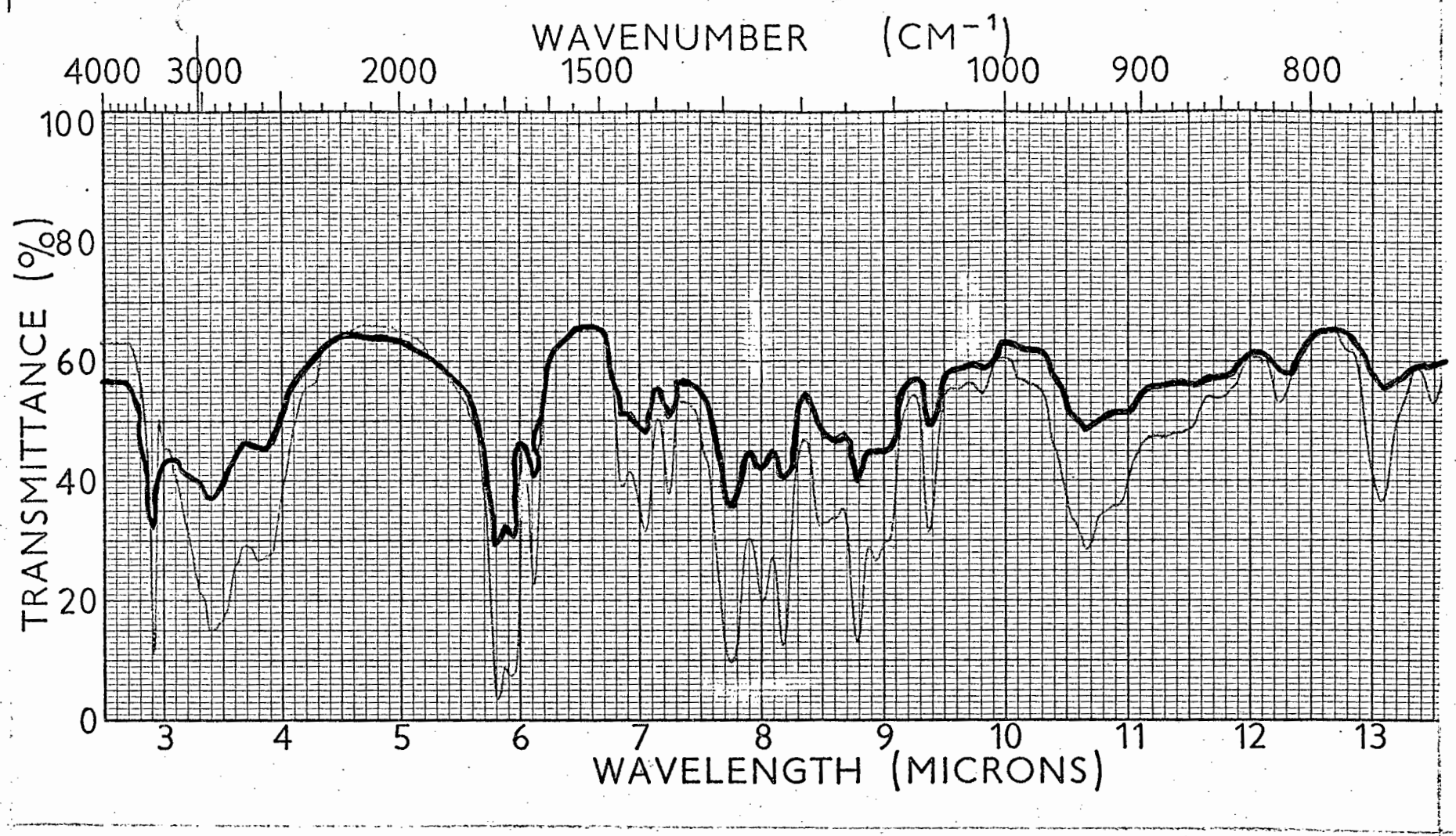


Fig. 1. APPARATUS USED FOR THE GRIGNARD REACTIONS



SAMPLE : Senecic Acid and Isomers.

Red Line : Sublimed Product.

Black Line : Residue.

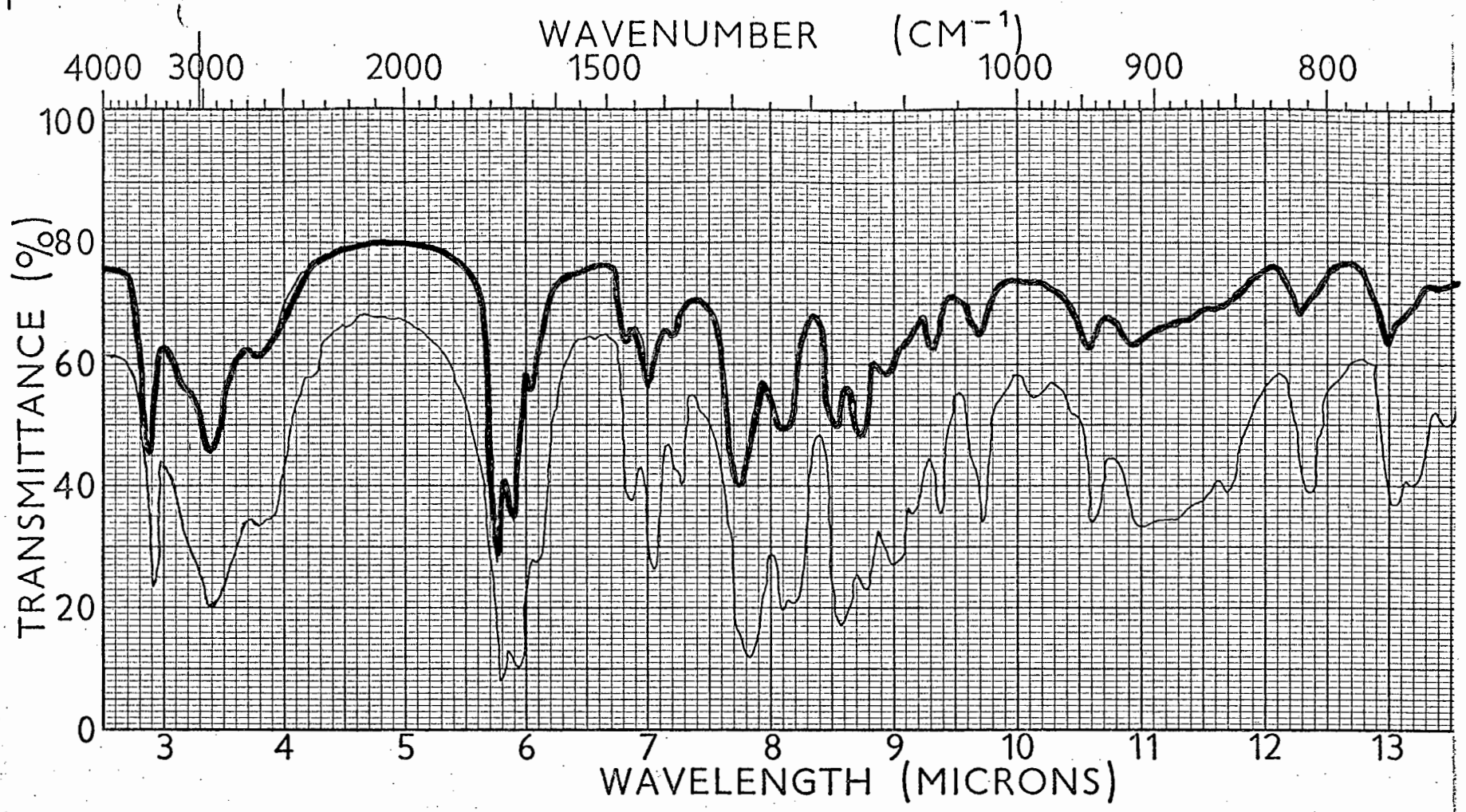
Phase : Solid.

Scan Speed : Fast.

Slit : Normal.

Operator : G. P.

Appendix I.



SAMPLE : Integerrinecic Acid.

Red Line : Integerrinecic Acid, authentic.

Black Line : Integerrinecic Acid, from conversion.

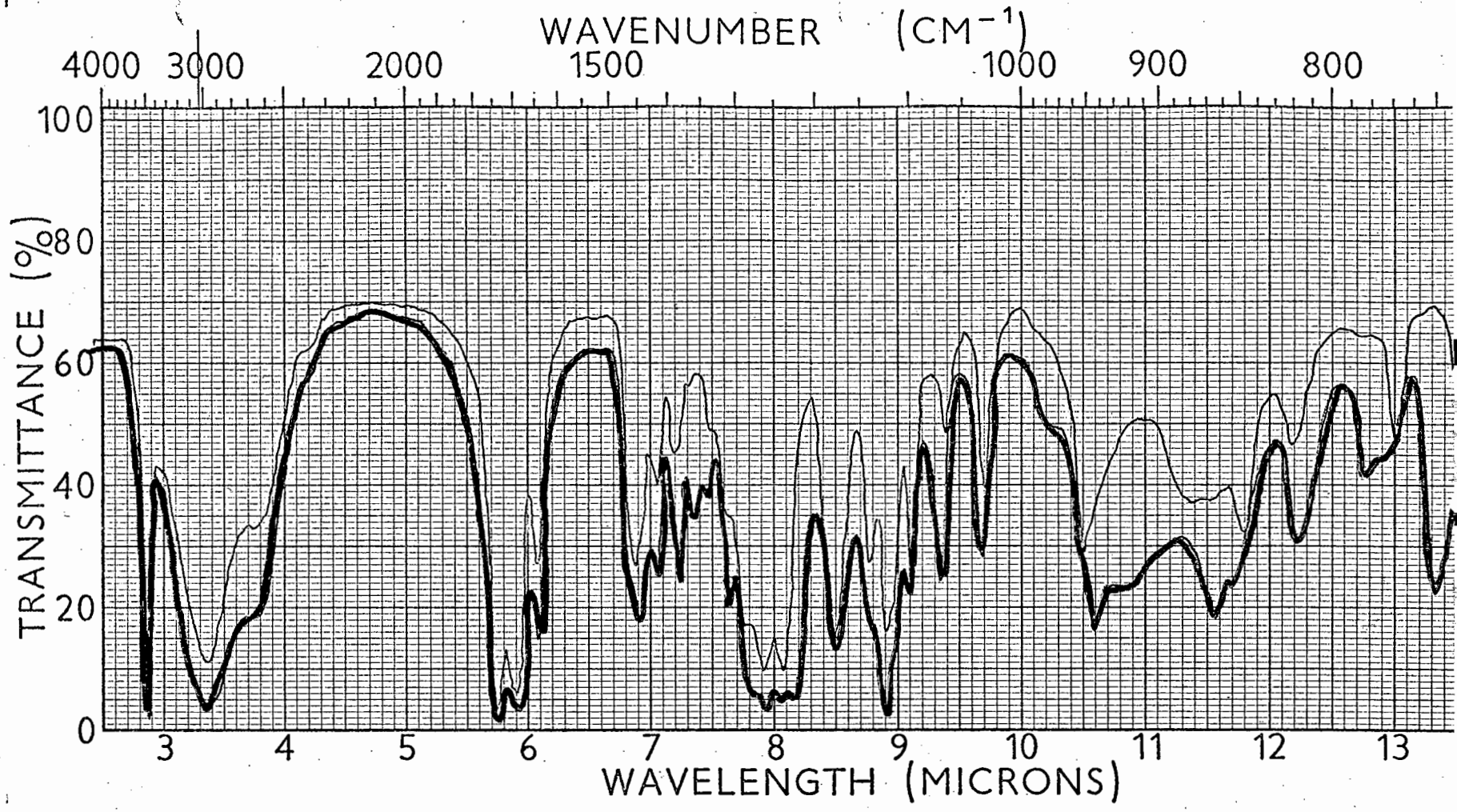
Phase : Solid.

Scan Speed : Fast.

Slit : Normal.

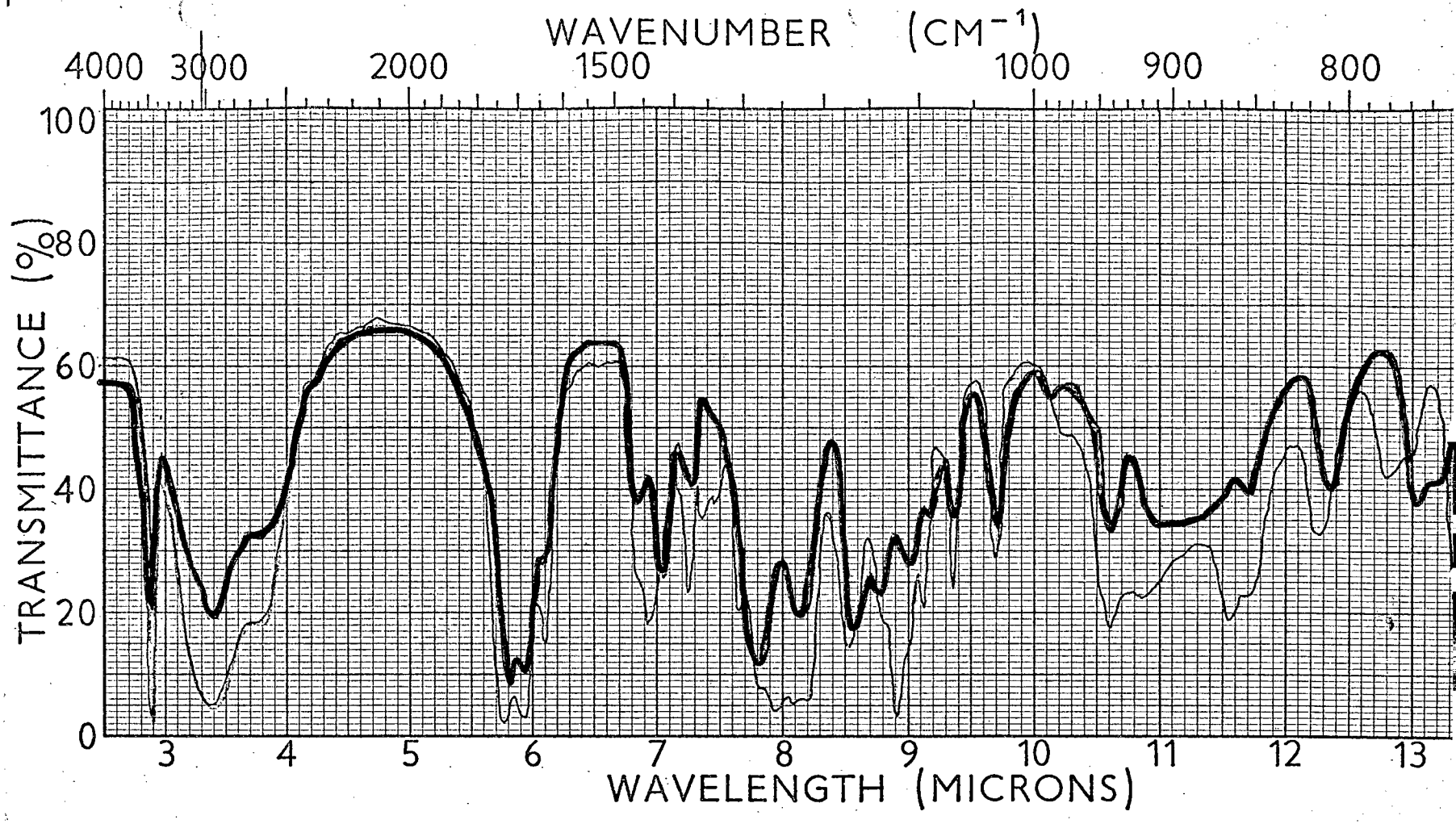
Operator : G. P.

Appendix II.



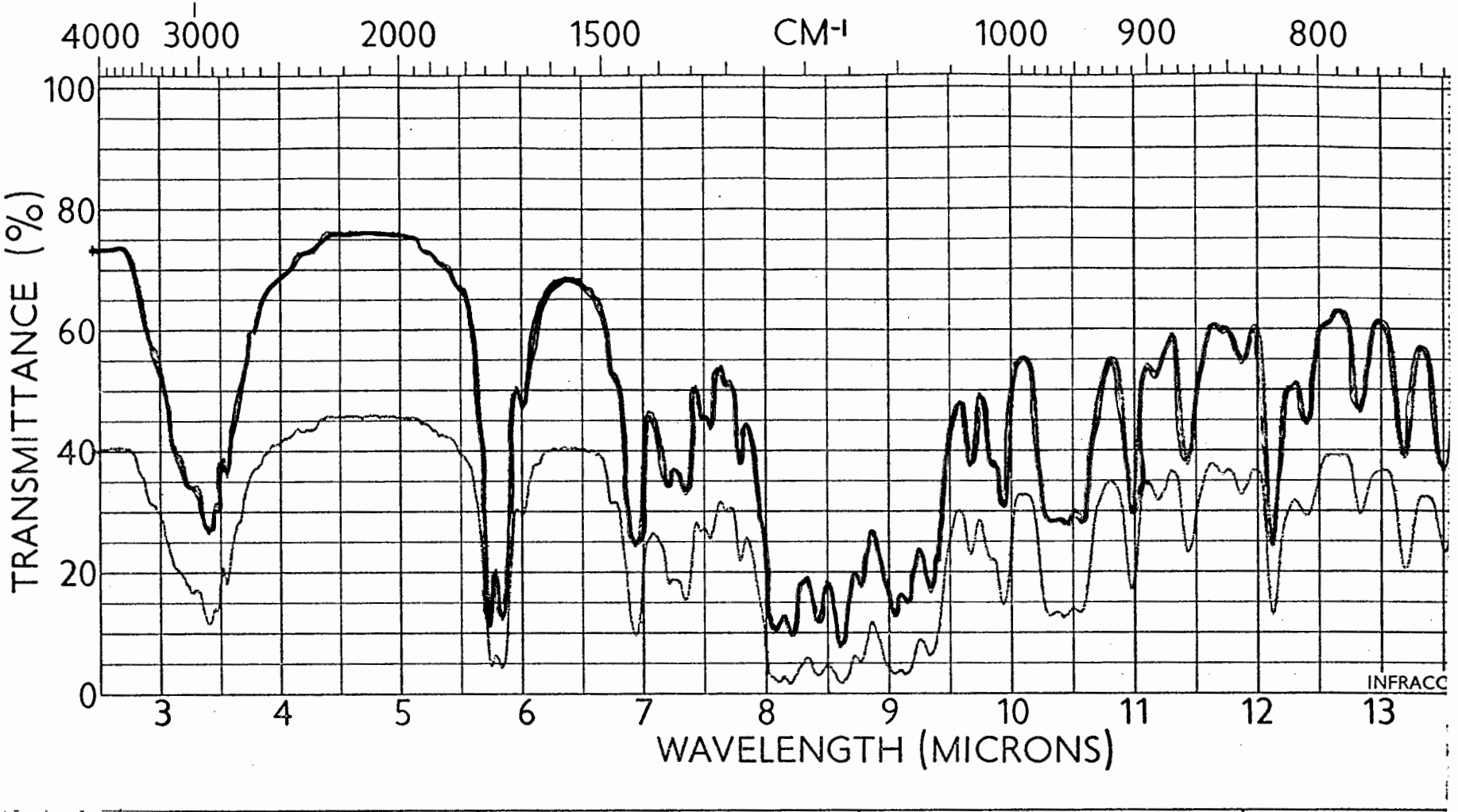
SAMPLE	:	Senecic and Diasenecic Acids.	Phase	:	Solid.
			Scan Speed	:	Fast.
Red Line	:	(+)-Senecic Acid.	Slit	:	Normal.
Black Line	:	(+)-Diasenecic Acid.	Operator	:	G. P.

Appendix III.



SAMPLE	:	Senecic and Integerrinecic Acid.	Phase	:	Solid.
			Scan Speed	:	Fast.
Red Line	:	Senecic Acid.	Slit	:	Normal.
Black Line	:	Integerrinecic Acid.	Operator	:	G. P.

Appendix IV.



SAMPLE : Senecionine.

Red Line : Senecionine, authentic.

Black Line : Senecionine, from conversion.

Phase : Solid.

Scan Speed : Fast.

Slit : Normal.

Operator : G . P.

Appendix V.

B I B L I O G R A P H Y

1.	Kropman, M., and F.L. Warren.	J.Chem.Soc.		2852 (1949).
2.	Baker, E.G.	J.Amer.Chem.Soc.	75	4631 (1953).
3.	Danilova, A.V., and Konovalova.	Doklady acad., Nank, S.S.S.R.	73	315 (1950).
4.	Culvenor, J., and T.A. Geissman.	J.Amer.Chem. Soc.	83	1947 (1961).
5.	Christie, S.M.H., M.Kropman, L.Novellie and F.L.Warren.	J.Chem.Soc.		1703 (1949).
6.	Kropman, M., and F.L. Warren.	J.Chem.Soc.		2852 (1949).
7.	Kropman, M., and F.L. Warren.	J.Chem.Soc.		700 (1950).
8.	Manske, R.H.F.	Canad.J.Res. Chem.Abstracts	 26	17B1 (1939). 1609 (1932).
9.	Fridrichson,J.,A.Mc L. Mathieson and J,D,Suter.	Tetrahedron Letters	23	35 (1960).
10.	Kropman,M., and F.L. Warren.	J.Chem.Soc.		2852 (1949).
11.	Richardson, M.F., and F.L.Warren.	J.Chem.Soc.		452 (1943).
12.	Schlosser, F.D., and F.L.Warren.	J.Chem.Soc.		5707 (1965).
13.	Adams, R. and B.L. van Duuren.	J.Amer.Chem. Soc.	75	4631 (1953).
14.	Cahn, R.S., C.K.Ingold and V.Prelog.	Experientia.	XII-3,	81 (1956).
15.	Reimer, M., and H.R. Downes.	J.Amer.Chem. Soc.		945 (1921).

16.	Kropman,M., and F.L. Warren.	J.Chem.Soc.		2854 (1949).
17.	Cram,D.J., and F.A.A. Elhafez.	J.Amer.Chem.Soc	74	5828 (1952.).
18.	Schlosser, F.D. and F.L.Warren	J.Chem.Soc.		5707 (1965).
19.	Waal, H.L.De	J.Vet.Science and Animal Industry.	14	433 (1940).
20.	Mattocks, M.	By direct communication with Prof.F.L.Warren.		
21.	Mattocks,M.	By direct communication with Prof.F.L.Warren		
22.	Edwards, J.et al	J.Org.Chem.	31	2282 (1966).
23.	Culvenor,J., and L.W. Smith.	Austral.J.Chem.		in press.
24.	Leonard, N.J.	The alkaloids (Ed.) Manske.	3	96
25.	Orekhov, A. and V. Tidebel	Ber.detsch.chem. Ges.	68	650 (1935).
26.	Orekhov, A. and R.A.Konovalova, V. Tidebel.	Ber.detsch.chem. Ges.	68	1886 (1935).
27.	Waal, H.L.,De	J.Vet.Sci.Animal Ind.	15	241 (1940).
28.	Richardson, M.F., and F.L.Warren.	J.Chem.Soc.		452 (1943).
29.	Danilova, A.V., and R.A.Konovalova.	J.Gen.Che.(USSR)	20	1921 (1950).
30.	Grandval,G., and H.Lajoux.	Bull.Soc.chim. France.	13	942 (1895)
31.	Barger, G., and J.J. Blackie.	J.Chem.Soc.		743 (1936).

32.	Briggs, L.H., J.L. Mangan and W.E.Russell.	J.Chem.Soc.		1891 (1948).
33.	Danilova, A., L.Utkin, G.V., Kosyrjewa and I. Syrnewa.	J.Gen.Chem.(USSR)	29	2432 (1959).
		Chem.Abstracts.	54	9980 (1960).